Chloro- and Bromo-Tryptophan Analogs as Phosphorescent Probes in Proteins

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quantum yield and photostability and the sorting capability provides the ultimate tool for accelerating the development of next generation RFPs.

1052-Pos Board B838

Heterogeneity and Kinetics of FRET-Based Ca^{2+} and Zn^{2+} Sensors in HeLa Cells

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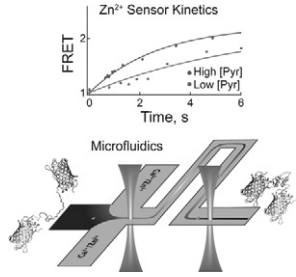
FRET-based genetically encoded metal-ion sensors coupled with fluorescence microscopy provide an important tool for real-time imaging of metal-ion homeostasis. The heterogeneity and *in vivo* kinetics are important sensor properties, yet are less explored because of lacking available experimental methods. We present a high-throughput microfluidic method capable of characterizing the FRET kinetics of metal-ion sensors in mammalian cells, at a throughput of 15 cells/sec with a time window of a few ms to several seconds. Using this method, we screened the HeLa cell Zn^{2+} sensor library and measured the FRET distribution at single cell level. We also report the quantitative high-throughput cellular-level heterogeneity measurement of cell populations, the cluster map of the Zn^{2+} sensor FRET change resolves 4 subpopulations with different metal response. Finally, we measured the *in vivo* sensor kinetics induced by changes in $[Zn^{2+}]$ and $[Ca^{2+}]$. We observed a > 30-fold rate difference between the extracellular and intracellular sensors, and the kinetics are ionophore-dependent.



1053-Pos Board B839

Phasor Diagram Analysis of Telomeric G-Quadruplex Stability and Heterogeneity

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Telomeres function to protect the end of linear chromosomes from damage and degradation. Human telomeres consists of several kilobases of the tandem repeat d(TTAGGG), which terminate in a 3'-single stranded overhang capable of forming G-Quadruplex structures. Telomeric G-Quadruplexes have potential roles in the maintenance of telomere integrity and the control of telomerase activity, an enzyme responsible for elongation of the telomeric overhang that is over expressed in a wide variety of cancers. Investigation of intramolecular G-Quadruplexes corresponding to the human telomere is complicated by the formation of multiple rapidly interconverting conformations. The multiple telomere G-Quadruplex conformations are identified by unique folding geometries containing distinct connecting loop regions.

Time-resolved fluorescent measurements have been conducted to characterize the stability and heterogeneity of the telomeric G-Quadruplex forming sequence $d(AGGG(TTAGGG)_3)$. The fluorescent adenine analog, 2-aminopurine, served as an internal fluorescent probe of G-Quadruplex conformation and was used to specifically monitor loop regions. 2-aminopurine demonstrates a complex fluorescent decay upon incorporation into nucleic acid polymers that can be simplified by transformation of time-resolved data to an (S,G) phasor diagram. Phasor diagrams were used to monitor G-Quadruplex formation upon addition of NaCl or KCl and elucidated a unique KCl-dependent transition. The digestion of G-Quadruplex structure by a single-strand specific nuclease and acrylamide quenching are also evaluated through phasor diagrams. This work demonstrates the utility of phasor diagrams for the study of nucleic acid systems and the ability to sensitively monitor complex fluorescent decays that may complicate analysis through nonlinear regression techniques. *Supported by awards RO1CA35635 and RO1GM077422 from the NCI and NIGMS.*

1054-Pos Board B840

Phasors Against Artifacts in Low-Frequency Phase Fluorometry and TCSPC

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The long fluorescence intensity decays exhibited by Ruthenium metal complexes are used to monitor reduced oxygen conditions in a range of biomedical and environmental applications. Both low frequency phase and modulation fluorometry (MPF) and Time Correlated Single Photon Counting (TCSPC) were used to monitor these changes. Direct drive-current-modulation and pulsed inexpensive, bright visible Light Emitting Diodes were employed. Lifetime data collected at room temperature using fused-silica cuvettes were analyzed in the classical way and compared with the phasor approach. Phasor plots were used to further elucidate the effects and removal of artifacts related to LED spectral cleanliness, emission filter excitation light leakage, measurement range cutoff, line frequency harmonics interference.

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Chloro- and Bromo-Tryptophan Analogs as Phosphorescent Probes in Proteins

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Tryptophan phosphorescence spectroscopy is one of the most sensitive methodologies around to study the local protein structure. For proteins in buffer, the phosphorescence lifetime (τ_p) directly reports about the local flexibility of the site with τ_p ranging from (sub) milliseconds for Trp at a solvent exposed position to several seconds if the Trp side chain is embedded in a rigid protein core. Because of the long intrinsic τ_p of Trp, 6.5 s, the method is extremely sensitive for quenchers e.g. oxygen and its concentration needs to be reduced to a level of several nM. This extreme sensitivity for quenchers has prevented widespread use of Trp phosphorescence in molecular biology. Substitution of the indole ring with a heavy atom is expected to significantly reduce the intrinsic τ_p, and thus the sensitivity for quenchers. We measured intrinsic τ_p values of 400 ms for 6-chloroindole, 13 ms for 6- bromoindole and 38 ms for 5-bromoindole, demonstrating the potential of these Trp analogs as phosphorescence probes in proteins. A straightforward approach to produce proteins labeled with these analogs was not available. We developed before a Trp auxotrophic strain of *Lactococcis lactus* able to incorporate Trp analogs in proteins which *E. coli* Trp auxotrophs do not translate. However, this *L. lactus* strain was found not able to translate bromo- or cloro-substituted Trp analogs. To overcome this, the aminoacyl t-RNA Trp synthetase enzyme from *L. lactus* was cloned and overexpressed in *L. lactus* together with a single-Trp containing target protein. This approach resulted in the efficient incorporating (87-91%) of the halogenated Trp analogs and a high protein yield (25-50 mg/L culture). Details of the methodology are presented as well as the spectroscopic characterization of the chloro- and bromo-Trp containing proteins.

1056-Pos Board B842

Using Metallic Nanoparticles for Nanometer-Resolution Optical Imaging in Living Cells: Photophysical Properties and Applications

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Labeling cells and tissues with fluorescent probes such as organic dyes and quantum dots (QDots) is a widespread and successful technique for studying molecular dynamics both in vitro and in vivo. However, those probes usually suffer from photophysical/photochemical processes, such us blinking and photobleaching, limiting their utilization. The main challenges in probe design are to improve single molecule fluorescence detection, and to provide higher stability against photobleaching. In the last few years, metallic nanoparticles (NPs) of various sizes, shapes, and materials have been used as new labels for cellular microscopy. This is in part because, unlike common organic dyes and QDots, metallic NPs do not bleach or blink upon continuous illumination, are extremely stable, very bright and their luminescence spans over the visible spectrum. Their excellent optical and chemical properties make them an attractive contrast agent for cell imaging both in vitro and in vivo. In this work we show some of their unique properties, and examples that illustrate their capabilities when used as probes in a single particle tracking experiment.

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1057-Pos Board B843

Intracellular Dynamics of Lysosomes Following the Disruption of Lysosome Associated Membrane Proteins (LAMP)

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The intracellular degradation of extracellular cargo is a process crucial to maintaining cellular health. In the classic model, degradation occurs inside the lysosome. However an alternative pathway has been proposed in which degradation occurs inside the late endosome, with the lysosome serving as an enzyme-storage vesicle. To test this new model, we have disrupted two key lysosomal proteins, LAMP1 and 2, in intact live cells using endoglycosidase H (Endo H). Following Endo H treatment, lysosome mobility was measured using live cell imaging and single particle tracking. Enzymatic activity was measured using fluorogenic substrates. We found that Endo H treatment alters the morphology and mobility of the lysosome and leads to alternate degradation pathways within the cell.